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SPECIES BOUNDARIES AND GLOBAL BIOGEOGRAPHY OF THE *ALEXANDRIUM TAMARENSE* COMPLEX (DINOPHYCEAE)[1]

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***Alexandrium catenella* (Whedon et Kof.) Balech, *A. tamarense* (M. Lebour) Balech, and *A. fundyense* Balech comprise the *A. tamarense* complex, dinoflagellates responsible for paralytic shellfish poisoning worldwide. The relationships among these morphologically defined species are poorly understood, as are the reasons for increases in range and bloom occurrence observed over several decades. This study combines existing data with new ribosomal DNA sequences from strains originating from the six temperate continents to reconstruct the biogeography of the complex and explore the origins of new populations. The morphospecies are examined under the criteria of phylogenetic, biological, and morphological species concepts and do not to satisfy the requirements of any definition. It is recommended that use of the morphospecies appellations within this complex be discontinued as they imply erroneous relationships among morphological variants. Instead, five groups (probably cryptic species) are identified within the complex that are supported on the basis of large genetic distances, 100% bootstrap values, toxicity, and mating compatibility. Every isolate of three of the groups that has been tested is nontoxic, whereas every isolate of the remaining two groups is toxic. These phylogenetic groups were previously identified within the *A. tamarense* complex and given geographic designations that reflected the origins of known isolates. For at least two groups, the geographically based names are not indicative of the range occupied by members of each group. Therefore, we recommend a simple group-numbering scheme for use until the taxonomy of this group is reevaluated and new species are proposed.**

***Key index words: Alexandrium*; biogeography; *catenella*; *fundyense*; paralytic shellfish poisoning; phylogeny; *tamarense*; taxonomy**

***Abbreviations:* bp, base pair; ITS, internal transcribed spacer; PSP, paralytic shellfish poisoning**

Paralytic shellfish poisoning (PSP) is a potentially fatal disorder caused by ingesting shellfish that contain high levels of neurotoxins called the saxitoxins. The parent compound, saxitoxin, and its congeners are produced by phytoplankton and accumulated in the tissues of filter-feeding shellfish (Taylor et al. 1995). Dinoflagellates of the genus *Alexandrium* are the most numerous and widespread saxitoxin producers and are responsible for PSP blooms in subarctic, temperate, and tropical locations (Taylor et al. 1995). The majority of toxic blooms have been caused by the morphospecies *A. catenella*, *A. tamarense*, and *A. fundyense* (Cembella 1998), which together comprise the *A. tamarense* species complex (Balech 1985).

Until 1970, PSP-causing dinoflagellates in the *A. tamarense* complex were known only from Europe, North America, and Japan (Hallegraeff 1993). By 2000, *A. tamarense* complex cells had been documented in the Northern and Southern hemispheres, adding South America, South Africa, Australia, the Pacific Islands, India, all of Asia, and the Mediterranean to the known range (Hallegraeff 1993, Abadie et al. 1999, Vila et al. 2001a). Concomitant with this substantial biogeographic expansion, the frequency of toxic *Alexandrium* blooms has also greatly increased (Anderson 1989, Hallegraeff 1993).

Because of the serious human health and economic impacts associated with PSP, this alarming increase has spurred the scientific community to investigate its origins. Four main theories have been proposed for this expansion (Anderson 1989): (i) The increase is actually an artifact of increased monitoring efforts uncovering previously undetected populations. (ii) Toxic populations have spread within regions through natural means, including transport in currents followed by the deposition of a dormant cyst stage. (iii) Environmental change,

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including eutrophication and global warming, may have caused toxic populations that were a normal, but minor, component of native flora to bloom to nuisance proportions. (iv) New populations have been initiated by human-assisted dispersal mechanisms, including ballast water transport and the importation of contaminated shellfish seed stock.

The recognition of previously undetected populations is possible because global awareness of HABs has risen, and monitoring efforts have expanded along with coastal fishing and aquaculture (Anderson 1989, Hallegraeff 1993). However, new populations of *A. tamarense* and *A. catenella* have also been detected in areas such as Thau Lagoon and Barcelona Harbor where established monitoring programs demonstrated a lack of these species prior to their recent appearance (Abadie et al. 1999, Vila et al. 2001a), and their subsequent spread (Penna et al. 2005).

Examples also can be found to support each of the remaining three hypotheses. For example, the source of *Alexandrium* populations in Argentina is unknown, but the spread of these organisms northward through Uruguay and Brazil is thought to be connected to the Malvinas current (Gayoso 2001, Persich et al. 2006). Human-assisted dispersal may explain the appearance of *A. catenella* populations in the Mediterranean (Lilly et al. 2002), and human alteration of the environment may be allowing that species to thrive and spread in its new habitat (Vila et al. 2001b).

Information like this is available for very few *A. tamarense* populations, and much of the evidence for other introductions is circumstantial (Gayoso 2001, Vila et al. 2001b, Persich et al. 2006). Yet if we are to prevent further spread of *A. tamarense*–complex cells, we must understand the causes for their current distribution. This undertaking requires investigation into two avenues: taxonomy and DNA phylogeny.

Unfortunately, taxonomy of the *A. tamarense* complex is contentious, with some researchers believing that the three morphologically defined species (morphospecies), *A. catenella*, *A. tamarense*, and *A. fundyense*, are true biological species (as defined by Mayr 1982). However, others contend that the morphological variations are not indicative of shared genetic heritage and instead may be variations within a single species (Anderson et al. 1994, Scholin et al. 1995). The morphospecies are highly similar in overall appearance, distinguished mainly by chain-forming ability, cell shape, and the presence or absence of a ventral pore between plates 1′ and 4′ (Balech 1995). *Alexandrium tamarense* strains can be either toxic or nontoxic and currently occur in most areas of the globe. *Alexandrium catenella* is distributed throughout much of the range of the *A. tamarense* complex, but unlike *A. tamarense*, all known strains of this morphospecies are toxic. *Alexandrium fundyense* is also always toxic, but its distribution is more limited, occurring mainly on the east coast of North America (Taylor 1984).

Because the morphological differences are slight and multiple morphospecies can co-occur (Taylor 1984, Anderson et al. 1994), researchers have searched for molecular evidence confirming or conflicting with the morphospecies identifications. The findings initially were confusing due to variation among geographic locations, supporting a distinction between *A. catenella* and *A. tamarense* in Japan (Sako et al. 1990, 1993, Adachi et al. 1994) but finding no clear differences in western or eastern North America and even uncovering morphological intermediates (Taylor 1984, Cembella and Taylor 1986, Hayhome et al. 1989, Anderson et al. 1994). Additionally, sexual reproduction was observed between clonal isolates of *A. tamarense* and *A. fundyense*, which yielded viable progeny capable of sexually producing an F2 generation (Anderson et al. 1994).

Each of these studies included multiple morphospecies, but all isolates were from a limited geographic region. In the mid-1990s, Scholin and Anderson (1994, 1996) and Scholin et al. (1994, 1995) published a series of studies comparing DNA sequences from ribosomal genes for *A. catenella*, *A. tamarense*, and *A. fundyense* strains from locations in eastern and western North America, Western Europe, Japan, and Australia along with several ballast water samples. Overall, these studies indicated that morphology is not a good indicator of evolutionary relationships within the *A. tamarense* complex.

Scholin et al. (1994) delineated five main phylogenetic clades, which they named after the origin of the majority of strains in each clade: North American, Western European, Temperate Asian, Tasmanian, and Tropical Asian. The fifth clade, Tropical Asian, consisted of a single sequence from isolate CU-13, which is now considered a member of a different species, *A. tropicale* Balech (Balech 1995, Lilly 2003). The Tasmanian clade also contained a single isolate. The North American clade contained examples of all three morphospecies, *A. catenella*, *A. tamarense*, and *A. fundyense*. In addition to strains from North America, this clade also contained the two *A. tamarense* strains that had been examined from Japan. The remaining strains from Japan were all *A. catenella*, and their sequences fell into a separate phylogenetic group, the Temperate Asian.

As yet, no consensus has been reached regarding the delineation of species within the *A. tamarense* complex, but the work of Scholin et al. (1994) has highlighted the utility of DNA sequences for biogeographic purposes. For example, nearly identical sequences were obtained for the D1-D2 divergent domains of LSU ribosomal DNA for strains of *A. catenella* from Japan, Australia, and the ballast water of a ship that operated exclusively between Japan and the same port in Australia from which the Australian cultures were isolated (Scholin et al.

1994). PSP has been recorded in Japan since the 1940s (Scholin et al. 1995), but the first recorded incident of PSP caused by *A. catenella* in Australia occurred in 1986 (Hallegraeff et al. 1988). Thus, the DNA sequences show that it is possible that *A. catenella* cells from Japan were introduced via ballast water to Australia (Scholin et al. 1995). Ribosomal DNA sequences have also been used in combination with fossil data to postulate a biogeographic history for the *A. tamarense* complex in the Northern Hemisphere (John et al. 2003).

The utility of DNA sequences to aid in determining the origin of toxic populations has spurred researchers around the world to investigate their local populations of *Alexandrium* using ribosomal DNA sequences (Adachi et al. 1996b, Yeung et al. 1996, Chen et al. 1999, Higman et al. 2001, Guillou et al. 2002, Kim and Kim 2002, Yeung et al. 2002, Kim et al. 2004a). One of the goals of this study was to incorporate the existing data from these and other sources into a global biogeographic analysis of the *A. tamarense* complex. This study also presents data for strains from additional areas, including South America, South Africa, the Russian coastline, and the Mediterranean, and increases the geographic coverage of strains from Europe and Asia. These data are compiled into a comprehensive view of the relationships among established and new populations. We reconstruct dispersal mechanisms in the *A. tamarense* complex and examine species boundaries and the possibility of cryptic species within the *A. tamarense* complex.

MATERIALS AND METHODS

Published DNA sequences. Some 126 D1-D2 LSU DNA sequences were available in GenBank for *A. catenella*, *A. tamarense*, and *A. fundyense* from multiple studies (Scholin et al. 1994, Haywood and MacKenzie 1997, Hansen et al. 2000, Godhe et al. 2001, Higman et al. 2001, Guillou et al. 2002, Kim and Kim 2002, Lilly et al. 2002, Usup et al. 2002, Yeung et al. 2002, Band-Schmidt et al. 2003, Kim et al. 2004a). Another 21 sequences were available from the literature (Scholin et al. 1994). Because this was a biogeographic study, sequences for which no geographic origins were given were removed. Numerous sequences (65) represented multiple PCR products from single isolates. These were reduced by removal of sequences resembling a previously known pseudogene (Guillou et al. 2002), recognized by an 87 base pair (bp) deletion. Remaining sequences for each isolate were aligned using Clustal X (Gibson et al. 1994) and checked in MacClade 4.05OSX (Maddison and Maddison 2000). Where sequences differed by single base changes they were condensed to a single sequence using base ambiguities. There remained a disproportionate number of isolates from the UK (24) and South Korea (68), from three regional studies (Higman et al. 2001, Kim and Kim 2002, Kim et al. 2004a). All UK sequences were aligned, and a distance matrix was calculated using PAUP 4.0b10 (Swofford 2002). The same was done for all Korean sequences. In cases where strains with identical sequences (a distance value of 0.00) came from the same geographic location or locations in very near proximity, one sequence was arbitrarily chosen, reducing the total number of taxa without losing variation. Additional sequences were pulled from GenBank for *A. affine* (Inoue et Fukuyo) Balech and *A. tamayavanichii* Balech, two closely related species (Lilly 2003), to serve as outgroup sequences. Table S1 (see the supplementary material) lists the strains used in this study with their morphospecies identification, locality of origin, GenBank accession number, and original citation. The geographic coverage of the strains is pictured in Fig. 1.

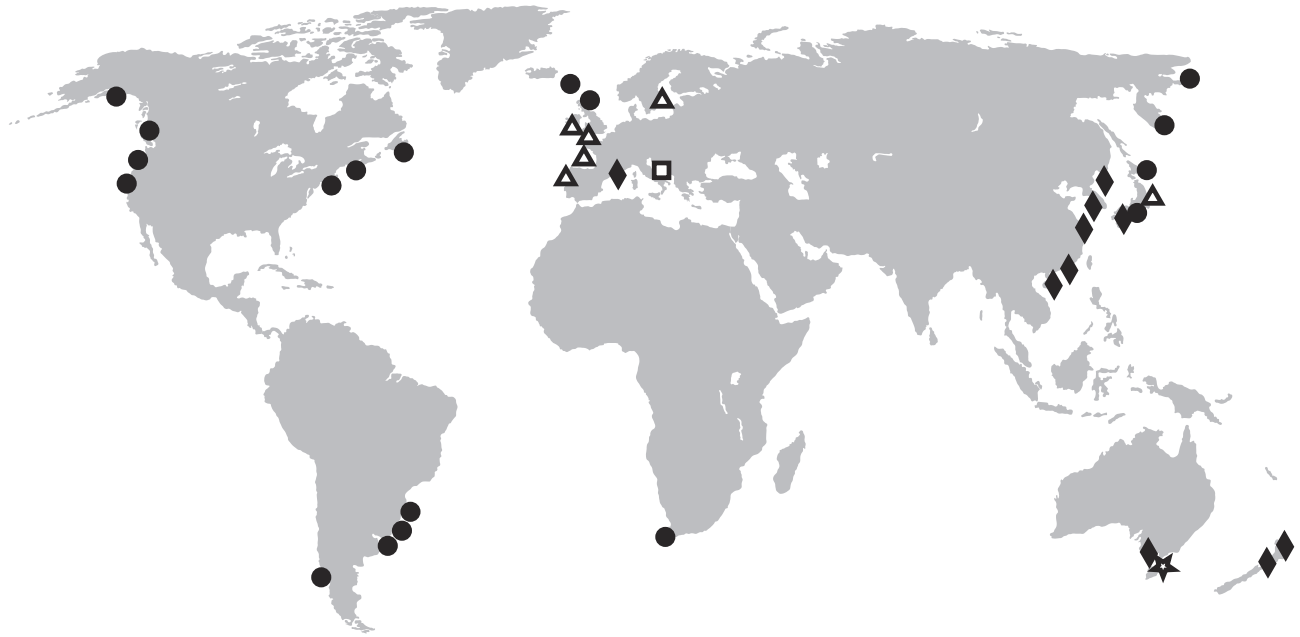

FIG. 1. The geographic origins of the strains used in this study. Each symbol may represent multiple isolates. See Table S1 (in the supplementary material) for a complete listing. Symbols refer to phylogenetic clades as depicted in Fig. 2. Black symbols represent toxic clades; white symbols represent nontoxic clades. Circle: Group I; square: Group II; triangle: Group III; diamond: Group IV; star: Group V.

Cultures. The existing sequences did not represent the entirety of the geographic range of the *A. tamarense* complex. Thus, 38 clonal cultures were obtained from South America (Chile, Uruguay, and Brazil), South Africa, eastern Russian (from Primorye to the Bering Sea), the Mediterranean Sea, and various areas throughout Europe, Asia, and the Pacific Islands. Three cultures of *A. tropicale,* a closely related species, and two of *A. affine* were also obtained. All cultures were maintained as described by Anderson et al. (1984), incubated at 15, 20, or 26°C, depending upon the natural environment for each strain.

DNA extraction. Because the multiple membranes and thecae of dinoflagellates can be difficult to rupture, midexponential phase cultures were subjected to osmotic shock to induce ecdysis. After centrifugation, the pellet was resuspended in 100 μL of the lysis buffer provided in the Qiagen DNeasy kit (Valencia, CA, USA). Samples were boiled for 25 min, frozen to −20°C, and thawed on ice. The DNeasy protocol was then followed as recommended by the manufacturer.

PCR amplification of D1-D2 LSU rDNA. Approximately 700 bp of divergent domains 1 and 2 (D1-D2) of the nuclear LSU rDNA were amplified from purified DNA or whole cell lysis products using PCR with D1R and D2C primers and 1–5 ng template, as previously described (Scholin and Anderson 1994). Products were purified in Qiagen MinElute PCR purification columns and stored in autoclaved deionized water at −20°C. Purified product concentration was determined relative to a DNA mass marker ladder (Low DNA Mass Ladder; Life Technologies, Carlsbad, CA, USA).

DNA sequencing. DNA sequencing used BigDye version 3.0 from Applied Biosystems Inc. (ABI, Foster City, CA, USA), in 6 μL volumes, with 20 ng template, 1.5 μM primer, and 1 μL BigDye. Reactions were run for 30 cycles of 96°C for 30 s; 50°C for 15 s; 60°C for 4 min, with a final hold at 4°C. Reactions were purified via isopropanol precipitation and then dried and stored at −20°C. Reactions were resuspended in HiDi Formamide (Applied Biosystems) and run on an ABI 3700. Templates were sequenced in duplicate in both directions.

DNA sequence analysis. Sequences were examined and assembled using ABI software and checked for base-calling accuracy. Sequences were aligned with published sequences and those of outgroup taxa using Clustal X (Gibson et al. 1994) and manually edited in MacClade 4.05OSX (Maddison and Maddison 2000).

Modeltest (Posada and Crandall 1998) was used to determine the substitution model and associated parameters. PAUP version 4.0b10 (Swofford 2002) was used for phylogenetic analyses. Neighbor-joining analysis was used to generate starting trees for maximum-likelihood analyses using model parameters generated in Modeltest. One thousand bootstrap replicates were run. Constrained analyses and Shimodaira–Hasegawa likelihood-ratio tests (Shimodaira and Hasegawa 1999) were preformed to test the hypotheses that (i) the three *A. tamarense* morphospecies, *A. catenella*, *A. tamarense*, and *A. fundyense*, each evolved independently and thus form monophyletic groups; and (ii) Groups I and IV (see Fig. 2), the toxic clades, are more closely related to one another than to the nontoxic clades.

RESULTS

The final data set included 110 strains distributed throughout the world. Notable gaps include the Indian Ocean, eastern Mediterranean, and parts of the west coast of Africa. Although we expect that *A. tamarense* strains do exist in these regions, cultures are currently not available. Five sequences of *A. affine* strains, four of *A. tamiyavanichii*, and four of *A. tropicale* were included as outgroup taxa. The remaining 97 sequences consisted of six *A. fundyense*, 27 *A. catenella*, and 64 *A. tamarense* sequences. Of 685 characters, 12 were excluded due to ambiguous alignment, 258 were parsimony informative, 46 were variable but parsimony uninformative, and 369 were constant.

Model testing. Modeltest estimated nucleotide frequencies as A = 0.2737, C = 0.1639, G = 0.2509, and T = 0.3115. The best fit to the data was obtained with six substitution types and rates (AC: 1; AG: 1.70891; AT: 0.5659; CG: 0.5659; CT: 2.5368; GT: 1), with no among-site rate variation and 46.19% of sites assumed to be invariable. These settings correspond to the GTR + I model (Rodriguez et al. 1990).

Tree topology. Two most-likely trees were produced of score −ln 2756.4879. The difference between the two trees consisted only of the placement of a single strain, DPC95b. One tree is shown in Fig. 2. In the tree not shown, DPC95b branched two nodes more basally. Neither placement was strongly supported by bootstrap analyses. The four *A. tropicale* sequences, two of which (CU-13 and CU-15) had been previously identified as Tropical Asian *A. tamarense* (Scholin et al. 1994), formed a monophyletic cluster closely related to the four *A. tamiyavanichii* sequences as previously observed (Lilly 2003).

The *A. tamarense* complex formed a monophyletic clade subdivided into five groups, Groups I, II, III, IV, and V, in Fig. 2. All groups were strongly supported with bootstrap values of 100%. Genetic distances among the groups were high, ranging from 6% to 11%, but distances within each group were low, ranging from complete identity to 2% divergence. Subdivisions within the groups were poorly supported, with two exceptions. First, the sequences Alex61-2 and UW4-1 were placed together on a long branch with 100% bootstrap support. Higman et al. (2001) interpreted these two sequences to represent pseudogenes because they obtained another, more typical, sequence from these same two clones (also included in Fig. 2). The second exception is the clustering of strains WKS-8, ACPP01, G. Hope 1, and TN9 within Group IV (bootstrap support = 95). The reason for this grouping is unclear. The strains come from three different geographic locations, Japan, South Korea, and Australia. The one unifying factor is that all were sequenced using the same method by Scholin et al. (1994). However, strain OF101 was also sequenced at that time and does not group with the other four.

Group I contained the sequences from Scholin et al.'s (1994) North American clade as well as sequences from South America, the Faroe Islands, Scotland, South Africa, and northern Asia (Russia, Japan, and Korea). In fact, the only major

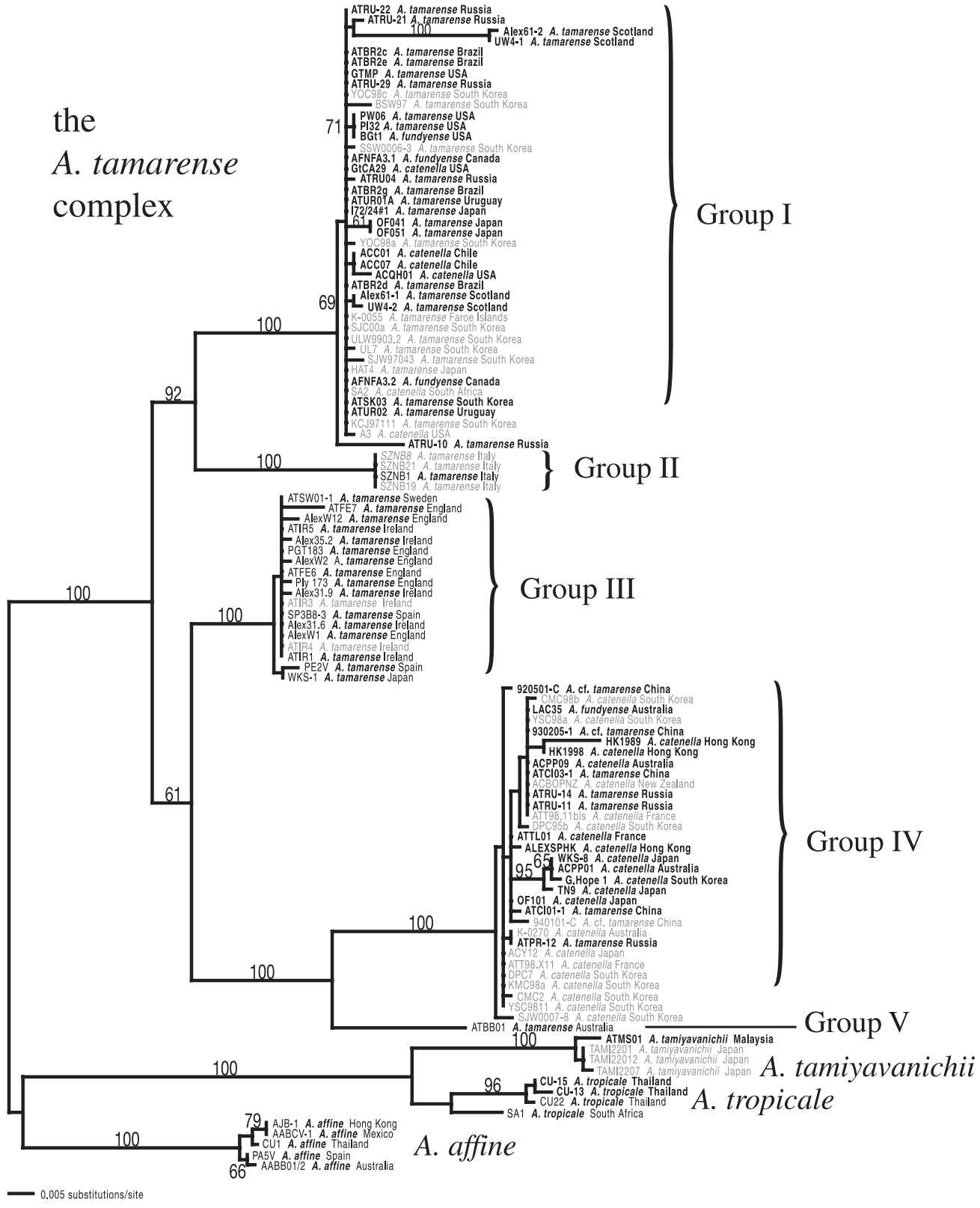

FIG. 2. One of two most-likely trees returned by maximum-likelihood analysis, score −ln 2756.4879. Strains are labeled with their original morphospecies designation. Strain numbers of toxic strains are indicated in bold type, while those of nontoxic strains are in nonbold type. Gray type is used where toxicity is unknown. See Table S1 (in the supplementary material) and Fig. 1 for the precise geographic origins of the strains.

geographic areas in which the *A. tamarense* complex occurs that were not represented in this group were southern Asia, Australia, and the Mediterranean. Group II consisted of four identical sequences from the Italian *A. tamarense* strains and is equivalent to the MED group identified by John et al. (2003). Group III was equivalent to Scholin et al.'s (1994) Western European clade, but not all of the strains were from the western portion of Europe. One isolate originated in the Baltic region (ATSW01-1); one was isolated from Tanabe Bay in Japan (WKS-1). Group IV contained the Temperate Asian group of Scholin et al. (1994) along with strains from China, Hong Kong, South Korea, Australia, Russia, and France. Many of the strains in Group IV did originate in temperate Asia; there were also representatives from Australia, New Zealand, and the Mediterranean Sea. Group V was identical to the Tasmanian group of Scholin et al. (1994) and contained only the single Tasmanian sequence.

Toxicity. It had been reported previously that each major clade within the *A. tamarense* complex consisted either entirely of toxic strains or entirely of nontoxic strains (Scholin et al. 1994, 1995). Although toxicity data were not available for all strains used in this study, the toxic versus nontoxic paradigm is upheld. All strains tested for toxicity in Groups I and IV were toxic, whereas tested strains in Groups II, III, and V were not toxic (Fig. 2). Toxic and nontoxic strains from close geographic proximity consistently fell out in separate groups. For example, the toxic strains UW4 and Alex61 from Scotland were placed within Group I, but the nontoxic strains from Cork Harbor, Ireland, and Weymouth and Plymouth, England, were placed within Group III. In Japan, nontoxic strain WKS-1 from Tanabe Bay also fell into Group III, but the toxic strains from Tanabe Bay, TN9 and WKS-8, were placed in Group IV (Fig. 2).

The two toxic groups, I and IV, are not most closely related to one another. A search constrained to place Groups I and IV as the nearest relatives resulted in significantly less-likely trees (−ln 2806.2277, $P = 0.002$). This finding implies that the ability to produce toxins has either been acquired or lost multiple times in the evolution of the *A. tamarense* complex.

Morphospecies relationships. The three morphospecies, *A. catenella*, *A. tamarense*, and *A. fundyense*, do not form monophyletic groups (Fig. 2). All three morphospecies are in Group I, although *A. tamarense* predominates. The three morphospecies are also in Group IV, although there is only one example of *A. fundyense* (LAC 35), and *A. catenella* is the most common morphotype. Groups II, III, and V consist entirely of *A. tamarense* strains. A search constrained to group each morphospecies as a monophyletic grouping returned four trees, all significantly longer than the most-likely tree (−ln = 3239.8004, $P < 0.000$). It is interesting to note that the three groups composed entirely of *A. tamarense* strains are also the three groups composed of nontoxic strains. *Alexandrium catenella* and *A. fundyense* always fall into one of the two groups containing toxic strains, as no nontoxic isolates are known for these two morphospecies.

DISCUSSION

Species definitions in the A. tamarense *complex.* Congruent with previous studies (Scholin et al. 1994, 1995), *A. catenella*, *A. tamarense*, and *A. fundyense* did not form separate clusters in our analyses. Although only *A. tamarense* was present in Groups II, III, and V, the three morphospecies intermingled in both Groups I and IV (Fig. 2). In some examples, *A. catenella*, *A. tamarense*, and *A. fundyense* have identical sequences over the D1-D2 LSU rDNA region. The lack of *A. catenella* and *A. fundyense* morphotypes in Groups II and V may be a sampling artifact due to the low number of samples. We thus confirm that *A. catenella*, *A. tamarense*, and *A. fundyense* are not valid species according to the phylogenetic species concept, as it requires all species to be reciprocally monophyletic (see Eldredge and Cracraft 1980, De Queiroz and Donoghue 1988).

While data are more sparse, we can also reject *A. catenella*, *A. tamarense*, and *A. fundyense* as true biological species because sexual reproduction and the production of viable progeny has been observed between isolates of *A. tamarense* and *A. catenella* (Sako et al. 1990, MacKenzie et al. 2004), and *A. tamarense* and *A. fundyense* (Anderson et al. 1994).

Alexandrium catenella, *A. tamarense*, and *A. fundyense* were defined originally according to the morphological species concept, in which species have constant, discrete characteristics. However, evidence from field studies and laboratory cultures indicates that the main distinguishing features, chain-forming ability, cell shape, and the ventral pore (Balech 1995), are neither discrete nor stable. Morphological intermediates have been observed in field samples (Taylor 1984, Cembella and Taylor 1986, Kim et al. 2002, Orlova et al. 2007), including chain-forming dinoflagellates with a ventral pore and cells with small, vestigial pores similar to those observed in the progeny of *A. tamarense* and *A. fundyense* matings (Anderson et al. 1994). Some clonal cultures maintain the ventral pore and chain-forming ability for many years (MacKenzie et al. 2004), but variation has also been observed in clonal cultures from geographic locations worldwide, with cells observed both with and without a ventral pore (E. L. Lilly, personal observation, Orlova et al. 2007, Gayoso and Fulco 2006). Other traits, such as chain-forming habit, thickness of thecae, and apical-antapical compression, are also variable in field samples and in culture. Leaw et al. (2005) recently combined morphological and molecular phylogenetics and determined that chain-forming ability and the presence

or absence of the ventral pore are homoplastic characters in the evolution of *Alexandrium* and might not be suitable for use as taxonomic markers (Leaw et al. 2005). Thus, *A. catenella*, *A. tamarense*, and *A. fundyense* also fail to meet the requirements of the morphological species concept.

Cryptic species. Some researchers have suggested that the entire *A. tamarense* complex is conspecific (Leaw et al. 2005), but this is not necessarily the case. Phylogenetically, the five reciprocally monophyletic, well-supported groups appear as valid species. The genetic distances among these groups (6%–11%) are comparable to the distances between uncontested species such as *A. tamiyavanichii* and *A. tropicale* (6%), and *A. tropicale* and *A. affine* (13%) (Lilly 2003). Within each of the five *A. tamarense* complex groups, genetic distances range from 1% to 2%, as is typical for haplotypes within a single species. Furthermore, techniques, such as amplified fragment length polymorphism, that discriminate strains within species work well within each phylogenetic group, but variability between groups is too high to be meaningful to distinguish species boundaries (John et al. 2004).

The high distances among the five groups and lack of intermediates are indicative of reproductively isolated species. Again, extensive mating trials have not been carried out, but preliminary data from matings between Groups I and IV indicate sexual incompatibility (D. M. Anderson, unpublished data). Thus, these five groups probably represent reproductively isolated cryptic species, such as have been proposed for *Scrippsiella* and *Peridinium* (Montresor et al. 2003, Kim et al. 2004b, Gottschling et al. 2005). John et al. (2003) noted that cryptic speciation is common in microbial eukaryotes, and the discovery of cryptic species of *Alexandrium* was predicted by MacKenzie et al. (2004). We recommend that more thorough mating trials and analysis of other molecular and morphological markers be conducted to investigate this hypothesis.

In the meantime, we recommend that the misleading morphospecies names and geographic group designations be set aside, and each of the five clades within the *A. tamarense* complex be considered a numbered group until such time as its independence as a unique species might be established and an appropriate name proposed. We realize that describing new cryptic species within the *A. tamarense* complex may be controversial and that some difficulty will arise from nomenclature changes. However, accurately identifying biologically and ecologically meaningful species will allow better understanding of *Alexandrium* physiology, ecology, and dispersal and may allow for better management strategies to be developed.

Biogeography. The five groups of the *A. tamarense* depicted in Figs. 1 and 2 are genetically distinct lineages and have different biogeographic histories. John et al. (2003) proposed a globally distributed ancestral population of *Alexandrium*, which subsequently diverged into two genetically distinct groups: the ancestors of Groups I and II, living in the waters off North America and in the tropical Atlantic, and the ancestors of Groups III, IV, and V in the northern portion of the Atlantic Ocean, the Tethys Sea, and the Indo-Pacific. John et al. (2003) further hypothesized that the ancestors of the nontoxic Group II evolved in the tropical Atlantic, becoming distinct from the Group I populations and later colonizing the Mediterranean. Nontoxic Group III is thought to have diverged from Group IV with the closing of the Tethys Sea. This theory separates the evolutionary history of the two major nontoxic groups and requires that toxicity either be gained independently twice (by Groups I and IV) or lost three independent times (by Groups II, III, and V).

Our most-likely trees also recover the separate origins of Groups II and III, and we agree with John et al. (2003) that the Group II population in the Mediterranean likely evolved in the Atlantic, arriving in the Mediterranean only with the most recent filling of the sea. This indicates that the evolutionary history of toxicity is complicated in the *A. tamarense* complex. Interestingly, the evolution of both Group II and Group III occurred in the eastern portion of the Atlantic Ocean, where similar environmental pressures may be responsible for the loss of toxicity in both groups.

Scholin et al. (1994) concluded that the Group I strains occurring in Japan were human introduced, given the lack of PSP records prior to 1948 (Anraku 1984). However, we document Group I populations in Korea, Kamchatka, and the Bering Sea, in addition to Japan and North America (Figs. 1 and 2). The broad distribution through the entire northern Pacific region indicates that this population is native to the region. Similarly, it has been suggested that the Group I isolates in Europe are human introduced (Higman et al. 2001) or natural populations (Medlin et al. 1998). Here, we have documented their presence in the Faroe Islands, an intermediate habitat that closes the gap between the two disjunct Group I populations in North America and Europe, supporting the natural dispersal hypothesis. Although it is not statistically significant, the ATRU-10 strain from the Bering Sea is basal to the entire Group I clade (Fig. 2), perhaps indicating an early Arctic population that later spread to both the Atlantic and Pacific oceans. Additional isolates from Arctic regions are being acquired to investigate this hypothesis.

There is also evidence of human-assisted dispersal in the Northern Hemisphere. For example, WSK-1 from Japan is a Group III nontoxic strain, and no intermediate habitats have been documented between Europe and Japan. The Group IV populations in the Mediterranean also appear to be introduced (Lilly et al. 2002). Monitoring programs in the Mediterranean have observed Group IV in

multiple locations along the French and Spanish coastlines (Vila et al. 2001b, Penna et al. 2005). Human alteration of harbors and beaches creates protected semienclosed environments that are perfect for these organisms, and Group IV appears to be thriving in its new home (Vila et al. 2001b).

In the Southern Hemisphere, we also see evidence of both natural and human-assisted dispersal. Reports of PSP from Chile date back to 1886 (Sengers 1908), and PSP in South Africa has been known since 1948 (Sapeika 1948), perhaps as early as 1888 [Gilchrist (1914) as cited in Sebastián et al. 2005], indicating that the Group I populations have been present longer than ballast water has been in use in commercial shipping. A natural dispersal mechanism, during a period when the climate was substantially cooler than it is today, such as during one of the recent ice ages, must account for the presence of *A. tamarense*–complex populations in both western South America and South Africa (see also Sebastián et al. 2005). However, eastern South America does not have a historic record of PSP. *Alexandrium tamarense* complex cells were first implicated in PSP in Argentina and Uruguay in 1980 (Carreto et al. 1985, Davison and Yentsch 1985) and in Brazil in 1996 (Odebrecht et al. 1997). Additionally, populations on the east coast of South America and Chile have identical D1-D2 LSU rRNA gene sequences, which may demonstrate that the eastern populations originated recently from Chile. Evidence indicates that *A. tamarense*–complex cells are spreading northward via natural current patterns (Persich et al. 2006). However, there must be an explanation for why the population was able to spread eastward recently even though it had historically not done so. Environmental change, in the form of global warming or coastal eutrophication, may hold the answer.

In the southern Pacific, most strains belong to the toxic Group IV, with the exception of the strain from Tasmania, which is nontoxic and comprises Group V. It is likely that Group V diverged from Group IV in the geologic past, and that Group V is endemic to the waters surrounding both the main island and Tasmania, while the Group IV strains are introduced (Scholin et al. 1994, 1995). This trend explains the reports of *Alexandrium*-like cells in the area prior to the 1980s without reports of PSP toxicity (Hallegraeff et al. 1991). We have also shown that the strains from New Zealand share identical D1-D2 sequence with strain ACPP09 from Port Phillip Bay, Australia, which supports MacKenzie et al. (2004), who note in their analysis that their data could indicate that the New Zealand population was recently introduced by human activities to the northern portion of the country through the major shipping ports. However, there are insufficient data to prove that the arrival of these organisms postdates the arrival of humans in New Zealand (MacKenzie et al. 2004). Regardless, Group IV is more widespread in the southern Pacific Ocean than previously thought and is thriving in this habitat.

CONCLUSIONS

The three morphospecies of the *A. tamarense* complex do not conform to the phylogenetic, biological, or morphological species definition and thus ought not to be considered valid species. Instead, five phylogenetic groups have been identified that are likely cryptic species, and we strongly recommend that these groups be evaluated for species-level status. An accurate taxonomy will be very useful for future monitoring efforts. Furthermore, we argue that geographically based names (e.g., North American ribotype, Western European ribotype) are no longer indicative of the range occupied by members of each group. Therefore, we recommend a simple group-numbering scheme for use until the taxonomy of this group is reevaluated and species names proposed. An extremely interesting and potentially useful feature of these groups is that each appears to contain either all nontoxic or all toxic strains. The reconstructed phylogeny and biogeography outlined in this paper indicate that both human-assisted and natural means have established the current distribution of the *A. tamarense* complex. This group of organisms is thriving in many coastal waters and could well be aided by coastal eutrophication, global warming, and the construction of enclosed harbors and beaches.

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Abadie, É., Amzil, Z., Belin, C., Comps, M.-A., Elziere-Papayanni, P., Lassus, P., Le Bec, C., Marcaillou-Le Baut, C., Nézan, É. & Poggi, R. 1999. *Contamination de l'étang de Thau par* Alexandrium tamarense*: Épisode de novembre à décembre 1998*. Ifremer, Sète, France, 44 pp.

Adachi, M., Sako, Y. & Ishida, Y. 1994. Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8S regions in Japanese *Alexandrium* species (Dinophyceae). *J. Phycol.* 30:857–63.

Adachi, M., Sako, Y. & Ishida, Y. 1996b. Identification of the toxic dinoflagellates *Alexandrium catenella* and *A. tamarense* (Dinophyceae) using DNA probes and whole-cell hybridization. *J. Phycol.* 32:1049–52.

Anderson, D. M. 1989. Toxic algal blooms and red tides: a global perspective. *In* Okaichi, T., Anderson, D. M. & Nemoto, T. [Eds.] *Red Tides: Biology, Environmental Science, and Toxicology*. Elsevier, New York, pp. 11–6.

Anderson, D. M., Kulis, D. M. & Binder, B. J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.* 20:418–25.

Anderson, D. M., Kulis, D. M., Doucette, G. J., Gallagher, J. C. & Balech, E. 1994. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeastern United States and Canada. *Mar. Biol.* 120:467–78.

Anraku, M. 1984. Shellfish poisoning in Japanese waters. *In* White, A. W., Anraku, M. & Hooi, K. K. [Eds.] *Toxic Red Tides and Shellfish Toxicity in Southeast Asia.* Southeast Asian Fisheries Development Center, Singapore, pp. 105–9.

Balech, E. 1985. The genus *Alexandrium* or *Gonyaulax* of the *A. tamarense* group. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates.* Elsevier, New York, pp. 33–38.

Balech, E. 1995. *The genus* Alexandrium *Halim (Dinoflagellata).* Sherkin Island Marine Station, Sherkin Island, Ireland, 151 pp.

Band-Schmidt, C. J., Lilly, E. L. & Anderson, D. M. 2003. Identification of *Alexandrium affine* and *A. margalefi* (Dinophyceae) from Mexico, using DNA sequencing and LSU rDNA-based RFLP-PCR assays. *Phycologia* 42:261–8.

Carreto, J. I., Negri, R. M., Benavides, H. R. & Akselman, R. 1985. Toxic dinoflagellate blooms in the Argentine Sea. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates.* Elsevier, New York, pp. 147–52.

Cembella, A. D. 1998. Ecophysiology and metabolism of paralytic shellfish toxins in marine microalgae. *In* Anderson, D. M., Cembella, A. D. & Hallegraeff, G. M. [Eds.] *Physiological Ecology of Harmful Algal Blooms.* NATO ASI Series. Springer, Berlin, pp. 381–403.

Cembella, A. D. & Taylor, F. J. R. 1986. Electrophoretic variability within the *Protogonyaulax tamarensis/catenelltic* species complex: pyridine linked dehydrogenases. *Biochem. Syst. Ecol.* 14:311–23.

Chen, Y.-Q., Qui, X.-Z., Qu, L.-H., Zeng, L.-M., Qi, Y.-Z. & Zheng, L. 1999. Analysis of molecular biogeographic marker on red tide toxic *Alexandrium tamarense* in the South China Sea. *Oceanol. Limnol. Sin.* 30:45–51.

Davison, P. & Yentsch, C. M. 1985. Occurrence of toxic dinoflagellates and shellfish toxin along coastal Uruguay, South America. *In* Anderson, D. M., White, A. W. & Baden, D. G. [Eds.] *Toxic Dinoflagellates.* Elsevier, New York, pp. 153–7.

De Queiroz, K. & Donoghue, M. J. 1988. Phylogenetic systematics and the species problem. *Cladistics* 4:317–38.

Eldredge, N. & Cracraft, J. 1980. *Phylogenetic Patterns and the Evolutionary Process: Method and Theory in Comparative Biology.* Columbia University Press, New York, 349 pp.

Gayoso, A. M. 2001. Observations on *Alexandrium tamarense* (Lebour) Balech and other dinoflagellate populations in Golfo Nuevo, Patagonia (Argentina). *J. Plankton Res.* 23: 463–8.

Gayoso, A. M. & Fulco, V. K. 2006. Occurrence patterns of *Alexandrium tamarense* (Lebour) Balech populations in the Golfo Nuevo (Patagonia, Argentina), with observations on ventral pore occurrence in natural and cultured cells. *Harmful Algae* 5:233–41.

Gibson, T., Higgins, D. G. & Thompson, J. 1994. *Clustal X: Improved Software for Multiple Sequence Alignment.* EMBL, Heidelberg, Germany.

Godhe, A., Otta, S. K., Rehnstam-Holm, A.-S., Karunasagar, I. & Karunasagar, I. 2001. Polymerase chain reaction in detection of *Gymnodinium mikimotoi* and *Alexandrium minutum* in field samples from southwest India. *Mar. Biotech.* 3:152–62.

Gottschling, M., Knop, R., Plotner, J., Kirsch, M., Willems, H. & Keupp, H. 2005. A molecular phylogeny of *Scrippsiella sensu lato* (Calciodinellaceae, Dinophyta) with interpretations on morphology and distribution. *Eur. J. Phycol.* 40:207–20.

Guillou, L., Nézan, E., Cueff, V., Erard-Le Denn, E., Cambon-Bonavita, M.-A., Gentien, P. & Barbier, G. 2002. Genetic diversity and molecular detection of three toxic dinoflagellate genera (*Alexandrium*, *Dinophysis*, and *Karenia*) from French coasts. *Protist* 153:223–38.

Hallegraeff, G. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32:79–99.

Hallegraeff, G. M., Bolch, C. J., Blackburn, S. I. & Oshima, Y. 1991. Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters. *Bot. Mar.* 34:575–87.

Hallegraeff, G., Steffensen, D. A. & Wetherbee, R. 1988. Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *J. Plankton Res.* 10:533–41.

Hansen, G., Daugbjerg, N. & Henriksen, P. 2000. Comparative study of *Gymnodinium mikimotoi* and *Gymnodinium aureolum,* comb. nov. (=*Gyrodinium aureolum*) based on morphology, pigment composition, and molecular data. *J. Phycol.* 36:394–410.

Hayhome, B. A., Anderson, D. M., Kulis, D. M. & Whitten, D. J. 1989. Variation among congeneric dinoflagellates from northeastern United States and Canada. I. Enzyme electrophoresis. *Mar. Biol.* 101:427–35.

Haywood, A. J. & MacKenzie, L. 1997. A phylogenetic analysis of LSU rDNA from New Zealand's *Alexandrium* species. GenBank. Available at http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db-nuccore&id-6465893 (accessed on September 20, 2007).

Higman, W. A., Stone, D. M. & Lewis, J. M. 2001. Sequence comparisons of toxic and non-toxic *Alexandrium tamarense* (Dinophyceae) isolates from UK waters. *Phycologia* 40:256–62.

John, U., Cembella, A., Hummert, C., Elbrächter, M., Groben, R. & Medlin, L. 2003. Discrimination of the toxigenic dinoflagellates *Alexandrium tamarense* and *A. ostenfeldii* in co-occuring natural populations from Scottish coastal waters. *Eur. J. Phycol.* 38:25–40.

John, U., Groben, R., Beszteri, B. & Medlin, L. 2004. Utility of amplified fragment length polymorphisms (AFLP) to analyse genetic structures within the *Alexandrium tamarense* species complex. *Protist* 155:169–79.

Kim, K. Y. & Kim, C. H. 2002. A molecular phylogenetic study on Korean *Alexandrium catenella* and *A. tamarense* isolates (Dinophyceae) based on the partial LSU rDNA sequence data. *Hangug Haeyang Haghoeji* 39:163–71.

Kim, C. J., Sako, Y., Uchida, A. & Kim, C. H. 2004a. Molecular phylogenetic relationships within the genus *Alexandrium* (Dinophyceae) based on the nuclear-encoded SSU rDNA D1-D2 sequences. *J. Korean Soc. Oceanogr.* 39:172–85.

Kim, E., Wilcox, L., Graham, L. & Graham, J. 2004b. Genetically distinct populations of the dinoflagellate *Peridinium limbatum* in neighboring Northern Wisconsin lakes. *Microb. Ecol.* 48:521–7.

Kim, K.-Y., Yoshida, M., Fukuyo, Y. & Kim, C.-H. 2002. Morphological observation of *Alexandrium tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and one related morphotype (Dinophyceae) in Korea. *Algae* 17:11–9.

Leaw, C. P., Lim, P. T., Ng, B. K., Cheah, M. Y., Ahmad, A. & Usup, G. 2005. Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and nuclear ribosomal gene sequence. *Phycologia* 44:550–65.

Lilly, E. L. 2003. *Phylogeny and Biogeography of the Toxic Dinoflagellate* Alexandrium. Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution Joint Program in Oceanography/Applied Ocean Science and Engineering, Woods Hole, Massachusetts, 226 pp.

Lilly, E. L., Kulis, D. M., Gentien, P. & Anderson, D. M. 2002. Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. *J. Plankton Res.* 24:443–52.

MacKenzie, L., de Salas, M., Adamson, J. & Beuzenberg, V. 2004. The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. *Harmful Algae* 3:71–92.

Maddison, D. R. & Maddison, W. P. 2000. *MacClade 4: Analysis of Phylogeny and Character Evolution.* Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Mayr, E. 1982. *The Growth of Biological Thought: Diversity, Evolution and Inheritance.* Harvard University Press, Cambridge, Massachusetts, 273 pp.

Medlin, L. K., Lange, M., Wellbrock, U., Donner, G., Elbrächter, M., Hummert, C. & Luckas, B. 1998. Sequence comparisons link toxic European isolates of *Alexandrium tamarense* from the

Orkney Islands to toxic North American stocks. *Eur. J. Protistol.* 34:329–35.

Montresor, M., Sgrosso, S., Procaccini, G. & Kooistra, W. H. C. F. 2003. Intraspecific diversity in *Scrippsiella trochoidea* (Dinophyceae): evidence for cryptic species. *Phycologia* 42:56–70.

Odebrecht, C., Mendez, S. & Garcia, V. M. T. 1997. Oceanographic processes and HAB in the subtropical southwestern Atlantic (26-36 degrees S). Poster represented at the VIII International Conference on Harmful Algae Vigo, Spain.

Orlova, T. Y., Selina, M. S., Lilly, E. L., Kulis, D. M. & Anderson, D. M. 2007. Interspecific morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the Russian Pacific coast. *Phycologia* 46:534–48.

Penna, A., Garcés, E., Vila, M., Giacobbe, M. G., Fraga, S., Luglie, A., Bravo, I., Bertozzini, E. & Vernesi, C. 2005. *Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean Sea. *Mar. Biol.* 148:13–23.

Persich, G. R., Kulis, D. M., Lilly, E. L., Anderson, D. M. & Garcia, V. M. T. 2006. Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil. *Harmful Algae* 5:36–44.

Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.

Rodriguez, F., Oliver, J. F., Marin, A. & Medina, J. R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* 142:485–501.

Sako, Y., Adachi, M. & Ishida, Y. 1993. Preparation and characterization of monoclonal antibodies to *Alexandrium* species. *In* Smayda, T. J. & Shimizu, Y. [Eds.] *Toxic Phytoplankton Blooms in the Sea.* Elsevier Science Publishing, Amsterdam, pp. 87–93.

Sako, Y., Kim, C., Ninomiya, H., Adachi, M. & Ishida, Y. 1990. Isozyme and cross analysis of mating populations in the *Alexandrium catenella/tamarense* species complex. *In* Granéli, E., Sundström, B., Edler, L. & Anderson, D. M. [Eds.] *Toxic Marine Phytoplankton.* Elsevier, New York, pp. 320–3.

Sapeika, N. 1948. Mussel poisoning. *S. Afr. Med. J.* 32:527.

Scholin, C. A. & Anderson, D. M. 1994. Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J. Phycol.* 30:744–54.

Scholin, C. A. & Anderson, D. M. 1996. LSU rDNA-based RFLP assays for discriminating species and strains of *Alexandrium* (Dinophyceae). *J. Phycol.* 32:1022–35.

Scholin, C. A., Hallegraeff, G. & Anderson, D. M. 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex' (Dinophyceae): dispersal in the North American and West Pacific regions. *Phycologia* 34:472–85.

Scholin, C. A., Herzog, M., Sogin, M. & Anderson, D. M. 1994. Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. *J. Phycol.* 30:999–1011.

Sebastián, R., Etheridge, S. M., Cook, P. A., O'Ryan, C. & Pitcher, G. C. 2005. Phylogenetic analysis of toxic *Alexandrium* (Dinophyceae) isolates from South Africa: implications for the global phylogeography of the *Alexandrium tamarense* species complex. *Phycologia* 44:49–60.

Sengers, P. A. 1908. Primera observación de una causa nueva de enfermidad del higago causando una hipertrofia y cirrosis atrofica consecutivas por excesividad funcional debido a abosorción de toxinas, y primera observación de esplenomgalia concomitante con hipertrofia de bazo en estas afecciones. *La Semana Médica* 20:117–9.

Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16:1114–6.

Swofford, D. 2002. *PAUP: Phylogenetic Analysis Using Parsimony.* Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Taylor, F. J. R. 1984. Toxic dinoflagellates: taxonomic and biogeographic aspects with emphasis on *Protogonyaulax*. *In* Ragelis, E. P. [Ed.] *Seafood Toxins.* American Chemical Society, Washington, D.C., pp. 77–97.

Taylor, F. J. R., Fukuyo, Y. & Larsen, J. 1995. Taxonomy of harmful dinoflagellates. *In* Hallegraeff, G. M., Anderson, D. M. & Cembella, A. D. [Eds.] *Manual on Harmful Marine Microalgae.* IOC Manuals and Guides No. 33. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 33.

Usup, G., Pin, L. C., Ahmad, A. & Teen, L. P. 2002. Phylogenetic relationship of *Alexandrium tamayavanichii* (Dinophyceae) to other *Alexandrium* species based on ribosomal RNA gene sequences. *Harmful Algae* 1:59–68.

Vila, M., Delgado, M. & Camp, J. 2001a. First detection of widespread toxic events caused by *Alexandrium catenella* in the Mediterranean Sea. *In* Hallegraeff, G. M., Blackburn, S. I., Bolch, C. J. & Lewis, R. J. [Eds.] *Harmful Algal Blooms 2000.* Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 8–11.

Vila, M., Garcés, E., Maso, M. & Camp, J. 2001b. Is the distribution of the toxic dinoflagellate *Alexandrium catenella* expanding along the NW Mediterranean coast? *Mar. Ecol. Prog. Ser.* 222:73–83.

Yeung, P. K. K., Kong, K. F., Wong, F. T. W. & Wong, J. T. Y. 1996. Sequence data for two large-subunit rRNA genes from an Asian strain of *Alexandrium catenella*. *Appl. Environ. Microbol.* 62:4199–201.

Yeung, P. K. K., Wong, F. T. W. & Wong, J. T. Y. 2002. Large subunit rDNA sequences from *Alexandrium catenella* strains isolated during harmful algal blooms in Hong Kong. *J. Appl. Phycol.* 14:147–50.

Supplementary Material

The following supplementary material is available for this article:

Table S1. DNA sequences used in this study. Strain designation, morphospecies, geographic origin, GenBank accession number, culture source, and original citation are given where available.

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